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Fluorescent Biosensors to Measure Endothelial Cell Responses to Fluid Shear Stress

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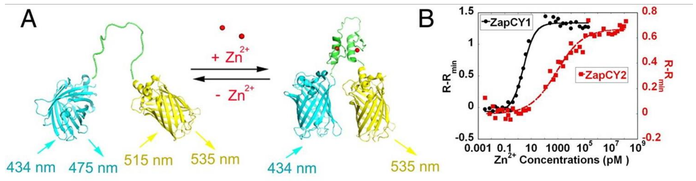
Introduction

- 10% of the human genome have ZN-binding proteins
- Atherosclerosis is correlated to vessel sites which experience oscillatory and reversing blood flow
- Changes in levels of zinc in different regions may alter signaling or other cellular processes (e.g. transcription, enzyme activity) leading to atherosclerosis

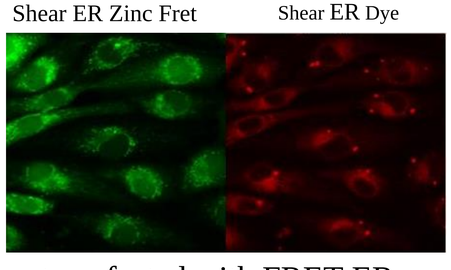
Measurement of Zinc

Our lab measures zinc inside the cell through the use of a FRET-based tension sensor that is inserted in the middle of a protein. The amount of FRET is proportional to the amount of zinc located in the cell.



- The sensor contains a Zap1 receptor in between two fluorescent proteins, cyan florecentprotein (CFP) and enhanced yellow fluorescent protein (YFP).
- Zn binding results in a conformational change that increases FRET from CFP to YFP.3
- This sensor can be genetically modified to result in its localization to specific subcellular organelles of the cell.
- Our lab uses four Zinc-FRET sensors will be used to measure zinc levels in the Nucleus, Golgi Body, ER or Cytoplasm.

Proof of Tension Sensor with Dye



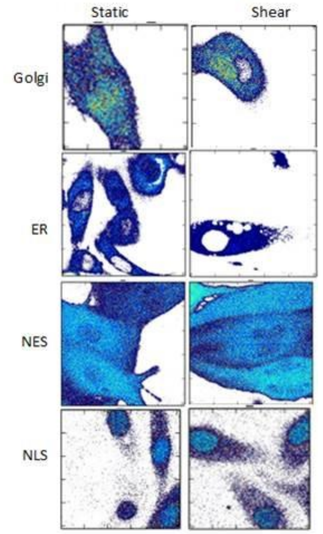
The cells were transfected with FRET-ER sensor, and then were died with and ER-Tracker dye. The dye and the FRET localized to the same area, proving the FRET-ER sensor localized correctly to the ER.

Localization of Zinc Sensor

- Zinc tension sensor localized to the Golgi, ER (endoplasmic reticulum), NES (nucleus excluding)
- Confocal microscopy showed that the zinc tension sensors located to the correct organelle.

Low Zinc — High Zinc

Zinc Localization Sensor



Conclusions

- Golgi, ER decrease with shear
- NES, NLS are the same with shear
- ER localizes properly, proved with dye

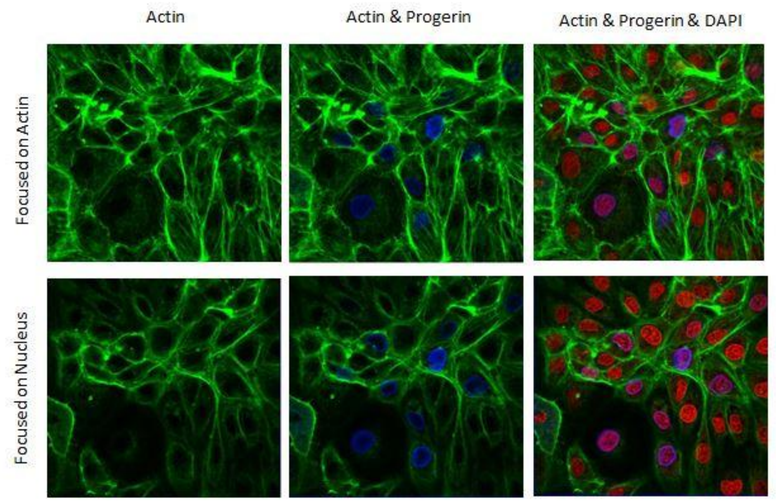
Nuclear Tension is Altered in Progeria

- Hutchinson-Gilford progeria syndrome (HGPS) causes a mutation in the nuclear lamin A
- Shown as laminar folding
- This same folding is caused by aging and is seen in the elderly

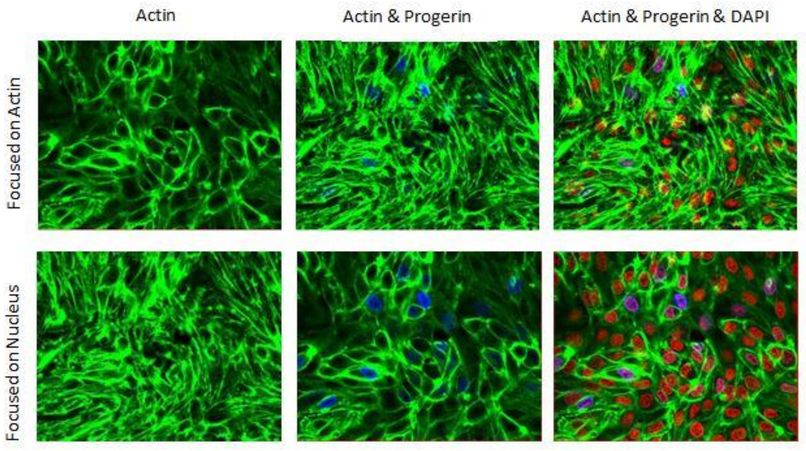
HGPS cells were grown in static culture. They were transferred to glass dishes where they were either left in static condition or sheared for 24 hours. The HGPS cells showed that the nucleus of the sheared cells had more ‘blebbiness’ in their nuclear lamin. The lamin was analyzed using anti-body staining to mark the nesprin, a linker of the cytoskeleton with the nucleoskeleton.

Actin Response to Shear Flow

Progeria Cells After 24h of Shear Flow



Static Cultured Progeria Cells After 24h



Conclusions

- Progeria cells show more ‘blebbiness’ under shear
- Static cultured progeria cells show actin that surrounds the nucleus on all axis
- Sheared progeria cells show that the actin does not cover over top of the nucleus
- Future direction is to add a nesbrin tention sensor so the force on the nucleus can be measured in HGPS cells during static and shear conditions

Acknowledgements

- VCU Undergraduate Opportunities Program Award